Role for yeast inhibitor of apoptosis (IAP)-like proteins in cell division

Anthony G. Uren*, Traude Beilharz†, Matthew J. O'Connell‡, Sarah J. Bugg‡, Rosemary van Driel§, David L. Vaux*¶, and Trevor Lithgow†

*The Walter and Eliza Hall Institute of Medical Research, Post Office Royal Melbourne Hospital, Victoria 3050, Australia; †Department of Biochemistry and Genetics, La Trobe University, Bundoora, Victoria 3083, Australia; ‡Research Division, Peter MacCallum Cancer Institute, St. Andrew's Place, East Melbourne, Victoria 3002, Australia; and §Baker Medical Research Institute, P.O. Box 6492, St. Kilda Road Central, Melbourne, Victoria 8008, Australia

ABSTRACT Inhibitors of apoptosis (IAPs) are a family of proteins that bear baculoviral IAP repeats (BIRs) and regulate apoptosis in vertebrates and *Drosophila melanogaster*. The yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* both encode a single IAP, designated *BIR1* and *bir1*, respectively, each of which bears two BIRs. In rich medium, *BIR1* mutant *S. cerevisiae* underwent normal vegetative growth and mitosis. Under starvation conditions, however, *BIR1* mutant diploids formed spores inefficiently, instead undergoing pseudohyphal differentiation. Most spores that did form failed to survive beyond two divisions after germination. *bir1* mutant *S. pombe* spores also died in the early divisions after spore germination and became blocked at the metaphase/ anaphase transition because of an inability to elongate their mitotic spindle. Rather than inhibiting caspase-mediated cell death, yeast IAP proteins have roles in cell division and appear to act in a similar way to the IAPs from *Caenorhabditis elegans* and the mammalian IAP Survivin.

Apoptosis is implemented by a mechanism highly conserved among metazoans. A number of mammalian cell death proteins resemble those from insects and nematodes both in structure and function, and some, such as members of the Bcl-2, caspase, and inhibitor of apoptosis (IAP) families are able to act in heterologous organisms (1), suggesting an ancient origin of the effector and control mechanisms of cell death.

Although examples of cell-suicide mechanisms have been described in single-celled organisms (reviewed in ref. 2), it is not yet known whether any similarity exists between the mechanisms of cell death in metazoans and unicellular organisms. In single-celled organisms, homologs of cell death molecules may be involved in cell death or may have unrelated roles. In either case, analysis of such proteins may reveal clues as to how they function. Although no homologs of Bcl-2 or caspases have been identified within single-celled organisms, by using low-stringency database searches we have identified homologs of IAP proteins in the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* (3). We designated these genes *BIR1* and *bir1*, respectively, because they bear a pair of BIR (baculovirus IAP repeat) motifs (4) within their amino termini.

IAP proteins are a family of cell death inhibitors identified in baculoviruses, where they prevent defensive apoptosis of the host cell and thereby promote viral replication (5). Function of these baculoviral IAPs is conserved, because they also were able to inhibit apoptosis of mammalian cells (6), and most of the cellular homologs of IAPs identified in *Drosophila* and vertebrates are also cell death inhibitors (7–10). Nevertheless, it is possible that some IAPs have other functions, as at least

one IAP from *Caenorhabditis elegans* is probably not involved in apoptosis but is required for cytokinesis during the first cell divisions after fertilization (11).

Here we describe the phenotype of *BIR1* and *bir1* deletion mutants and localization of the proteins they encode.

MATERIALS AND METHODS

S. cerevisiae **Culture and Transformation.** Isogenic wild-type strains JK9 (*Mat**a**/Matα, his4/his4, leu2/leu2, ura3/ura3, trp1/trp1*) and MH272 (*Mat**a**/Matα his3/his3, leu2/leu2, ura3/ ura3, trp1/trp1, ade2/ade2*) were used. Cells were grown in rich medium (YPD: 1% yeast extract/2% bactopeptone/2% glucose) at 30°C to late log phase and transferred to presporulation medium (YEPA: 1% yeast extract/2% bactopeptone/ 0.2 M potassium acetate) for a further 24 hr and then resuspended in sporulation medium (SM: 30 mM potassium acetate/0.3 mM raffinose), incubated for up to 12 days, and prepared for tetrad dissection (12) or microscopy.

PCR-mediated gene disruption (13) was used to delete the *BIR1* gene. A DNA fragment incorporating the complete *HIS3* gene from *S. cerevisiae* was amplified from plasmid DNA by PCR using oligonucleotide primers including 50 nt corresponding to the 5′ and 3′ ends of the *S. cerevisiae* BIR1 gene. The yeast strain MH272 was transformed with this construct, and His+ transformants were checked by Southern blotting to confirm the identity of the yeast strain YAU1 (*Mat**a**/Matα his3/his3, leu2/leu2, ura3/ura3, trp1/trp1, ade2/ade2, BIR1/HIS3::bir1*).

To create a homozygous mutant strain, YAU1 was sporulated, and after tetrad dissection, haploid Δbir1 strains of each mating type were identified and mixed on plates of rich medium. Zygotes then were identified and isolated. The homozygous diploid YAU11 (*Mat**a**/Matα, his3/his3, leu2/ leu2, ura3/ura3, trp1/trp1, ade2/ade2, HIS3::bir1/HIS3::bir1*) can sporulate, albeit poorly, confirming its diploid status.

Pseudohyphal Growth Experiments. YAU11 cells were transformed with gene fragments encompassing the *URA3* gene, the *LEU2* gene, the *ADE2* gene, and the *TRP1* gene to create the heterotrophic *bir1* mutant strain YAU1100 (*Mat**a**/ Matα, Δbir1/Δbir1*). The YAU1100 cells were transferred to nitrogen-poor SLAD medium (14) and compared with an isogenic, heterotrophic wild-type strain. Both the wild-type and *bir1* mutant strain grew with an elongated morphology characteristic of pseudohyphae, demonstrating that *BIR1* is not required in the signaling pathway for pseudohyphae formation. Some *S. cerevisiae* mutants maintain a constitutively active pseudohyphal state. This includes the *elm1* (14) and *tec1* (15) mutants. To test whether the *BIR1* gene could suppress this

---

signaling pathway, *elm1–1* and *tec1* mutants were transformed with the multicopy vector pYX212-BIR1. No change was observed in the cells overexpressing BIR1 relative to untransformed cells or cells transformed with the empty plasmid pYX212.

Electron Microscopy. *S. cerevisiae* cells were cultured for 28 hr in SM and then fixed in 1% paraformaldehyde/1% glutaraldehyde in 0.04 M phosphate buffer (PB), pH 6.8, and incubated for 5 min at 20°C and then 30 min on ice. For staining, cells were washed three times in PB, incubated in 1% sodium metaperiodate for 15 min, washed, and incubated in 50 mM ammonium chloride for 15 min at 20°C. The cells were postfixed at 20°C in 0.5% potassium permanganate, 5 min as suspension, and then fixed as a pellet for an additional 20 min at 20°C. After washing they were en bloc stained in 0.5% uranyl acetate overnight at 4°C. The stained cells dehydrated in 50, 70, and 95% ethanol and, finally, in three changes of 100% ethanol. Cells were infiltrated with LR White resin as follows: ethanol/resin (1:2) for 1 hr, rotating, ethanol/resin (1:1) for 1 hr, ethanol/resin (1:1) overnight, and then two changes of pure resin for 1 hr. Cells then were embedded and polymerized at 55°C for 2 days. Sections were cut and collected onto 0.25% formvar-coated grids. Uranyl acetate and lead citrate were used for contrasting, and the sections were examined by using a JEOL 1200EX transmission electron microscope.

S. pombe Culture and Spore Preparation. Strains were derivatives of 972 h⁻ and 975 h⁺. Wild-type diploids (*leu1–32/leu1–32 ura4-D18/ura4-D18 ade6-M210/ade6-M216* h⁺/h⁻) and haploids (*leu1–32 ura4-D18 ade6–704* h⁻) were used. Usual procedures for the propagation and manipulation of *S. pombe* were used (16). Large-scale sporulation cultures were expanded in yeast extract media plus amino acids and then transferred to Edinburgh minimal medium lacking a nitrogen source (ammonium chloride) plus amino acids. The resulting asci were digested at 37°C in glucuronidase (Sigma), washed extensively in water, and germinated in EMM plus amino acids.

S. pombe Constructs. The *bir1* knockout construct was assembled by PCR amplification of 1-kb arms surrounding the ORF, which then were subcloned into pBluescript. A *ura4* marker was subcloned between these arms. This construct was linearized and transformed into wild-type haploid and diploid cells by using a lithium acetate-transformation procedure.

Southern blots of DNA digested with *Hin*dIII and *Cla*I were used to screen for knockouts.

The *bir1* ORF lacking the predicted intron of the genomic sequence was amplified by PCR from an *S. pombe* cDNA library (17) and subcloned into the pRep3X expression vector (18). A version of the *bir1* ORF lacking a stop codon also was amplified by PCR and fused to the GFP(S65T) ORF, and this fusion was subcloned into pRep3X.

S. pombe Microtubule Staining. Germinated spores were fixed in 3.7% formaldehyde/0.25% glutaraldehyde and processed for microtubule staining as described (19) with a 1:100 dilution of primary antibody TAT-1 (a gift of Keith Gull, University of Manchester) and 1:100 dilution of Cy3-labeled anti-mouse IgG (Sigma).

RESULTS

***S. cerevisiae* and *S. pombe* Both Have Conserved BIR Motif Proteins.** Nucleic acid databases of the yeasts *S. cerevisiae* and *S. pombe* were searched for ORFs containing conserved residues characteristic of the BIR motifs of metazoan IAP proteins. A 954-aa ORF was identified from *S. cerevisiae* (YJR089w) encoding two BIR motifs near its N terminus (Y12 to I116 and D145 to F240) and a putative nuclear localization sequence (K386 to R393) (Fig. 1A). A similar ORF was identified in *S. pombe* (cosmids SPAC2C6.16 and SPCC962.02c). The predicted spliced product encodes a protein of 997 aa, which contains two N-terminal BIR motifs (R17 to A98 and Q117 to F193), a putative nuclear localization sequence (P288 to K295), and a putative nucleotide-binding domain (G622 to S629) (Fig. 1A). These yeast BIR motifs contain a large proportion of the key conserved residues common to all BIRs, including the invariant cysteine and histidine residues (Fig. 1B). In addition to having two BIR motifs at their N termini and being similar in length, they also share a conserved domain at their C termini (Fig. 1C).

Comparison of the BIRs of various IAPs place these yeast BIR motifs in a subfamily along with those of two *C. elegans* IAPs and the mammalian IAPs Survivin (20) and BRUCE (21) (Fig. 1B). BIR motifs from this class of IAPs are longer than other BIR motifs, because of the presence of larger, variable gaps between the universally conserved GLY and first CYS residues. Notably, a conserved intron is present at the
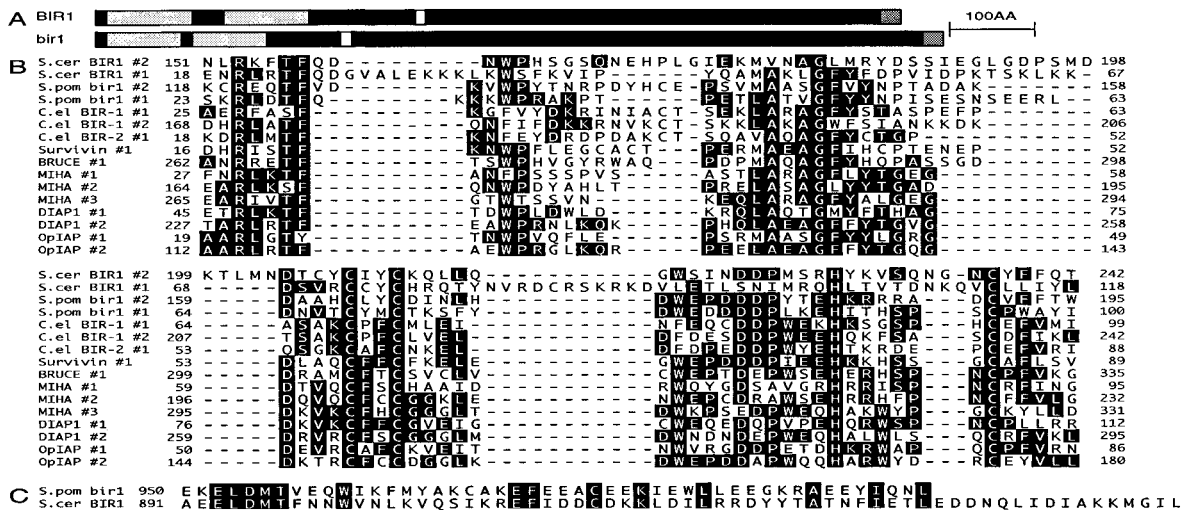

FIG. 1. BIR motif containing proteins of *S. cerevisiae* and *S. pombe*. (*A*) Domain structure of BIR1 and Bir1. BIR1 and Bir1 are similar in length and contain two BIR motifs at their N termini, a putative nuclear localization signal near their centers, and a conserved domain at their C termini. (*B*) The BIR motifs of BIR1 (*S. cerevisiae*), Bir1 (*S. pombe*), both *C. elegans* IAP homologs, Survivin (human), BRUCE (human), and anti-apoptotic IAPs such as MIHA (mouse), DIAP1 (*Drosophila*), and OpIAP (baculovirus) share a number of key conserved residues. IAPs that also contain RING finger motifs, such as MIHA and OpIAP, are more closely related to each other than they are to the longer class of BIR motifs. (*C*) Conserved residues within the C-terminal motif of BIR1 and Bir1.

same position just 5′ of the nucleotides encoding the invariant GLY residue within the BIRs in *S. pombe bir1* (BIR #2), both *C. elegans* IAPs, and *survivin* (Fig. 1*B*). The absence of a similarly placed intron within *BIR1* is not surprising, given the paucity of intron-containing genes in *S. cerevisiae*.

BIR1 Is Required for Both Efficient Meiotic Division and Spore Germination in *S. cerevisiae*. One copy of the ORF of *BIR* was deleted in diploid *S. cerevisiae* by homologous recombination. These diploid heterozygotes were induced to sporulate, and the growth phenotypes of the wild-type and Δ*bir1* haploid daughter cells were assayed. Although the *BIR1* gene was not required for vegetative growth of haploid cells, spore formation was delayed in the diploid heterozygotes, and less than 25% of these cells formed complete tetrads.

Homozygous mutant Δ*bir1*/Δ*bir1* diploid cells also underwent normal vegetative growth in rich medium (1% yeast extract, 2% peptone, 100 mg/liter adenine sulfate/2% dextrose), but their ability to form complete tetrads was diminished greatly. In wild-type cells some complete tetrads were visible after 1 day in sporulation medium (SM), and the process was largely complete after 3 days. In marked contrast, the mutant Δ*bir1*/Δ*bir1* cells did not generate complete tetrads until 6–12 days on sporulation medium, and even then these represented less than 5% of the culture (Fig. 2*A*).

When complete tetrads isolated from the Δ*bir1*/Δ*bir1* mutant cells were dissected and replated, more than 90% of the haploid Δ*bir1* spores failed to complete germination (Fig. 2*B*). 4′,6-Diamidino-2-phenylindole (DAPI) staining of complete tetrads invariably showed one nucleus per haploid spore, but partial aneuploidy of these nuclei cannot be ruled out (Fig. 2*C*). The mutant Δ*bir1* spores initiated mitosis, but died at the two- to five-cell stage.

Loss of *BIR1* Induces Inappropriate Pseudohyphal Development. In the absence of a rich nitrogen source, diploid *S. cerevisiae* cells begin either of two developmental programs: under conditions of starvation, they undergo meiosis and form a tetrad of spores, whereas if a rich carbon source such as glucose is readily available, they develop into pseudohyphae (22). Diploid Δ*bir1*/Δ*bir1* mutant cells were indistinguishable from wild-type diploids when grown in rich medium, except for the presence of a small proportion of cells in these cultures (<5%) that displayed characteristics of pseudohyphal development such as elongation of cells and unipolar cell division. The proportion of pseudohyphal cells was increased in cultures in presporulation or sporulation medium. After 12 days of culture in sporulation medium, pseudohyphal structures represented 10–20% of the culture (Fig. 2*D*). In contrast, parental wild-type cells did not develop pseudohyphae when cultured in rich, presporulation or sporulation medium.

Formation of pseudohyphae was not simply due to the failure of Δ*bir1*/Δ*bir1* cells to sporulate because pseudohyphal development of Δ*bir1*/Δ*bir1* cells was observed in rich (YPAD) cultures under conditions in which the corresponding wild-type cells neither arrested nor initiated sporulation. Furthermore, other sporulation defectives [e.g., Δ*tom22* cells, which are compromised in sporulation because of mitochondrial defects (23)] do not undergo pseudohyphal development as a default pathway when sporulation is blocked. Despite the inappropriate pseudohyphal development in Δ*bir1* mutants, neither loss of the *BIR1* gene nor overexpression of *BIR1* on a multicopy plasmid could suppress constitutive pseudohyphal growth in *elm1* or *tec1* yeast mutants (T.L., unpublished data).

Appearance of *bir1* Mutant Yeast by Electron Microscopy. Cultures of wild-type and Δ*bir1*/Δ*bir* cells were harvested after 28 hr in SM and were fixed, mounted, and sectioned for electron microscopy analysis. In wild-type samples the majority of cells (≈80%) contained a large vacuole in their center and a number of smaller lipid bodies and vesicles at their periphery (Fig. 3*A*), indicative of cells that have initiated meiosis I and are undergoing formation of the spore wall (24).
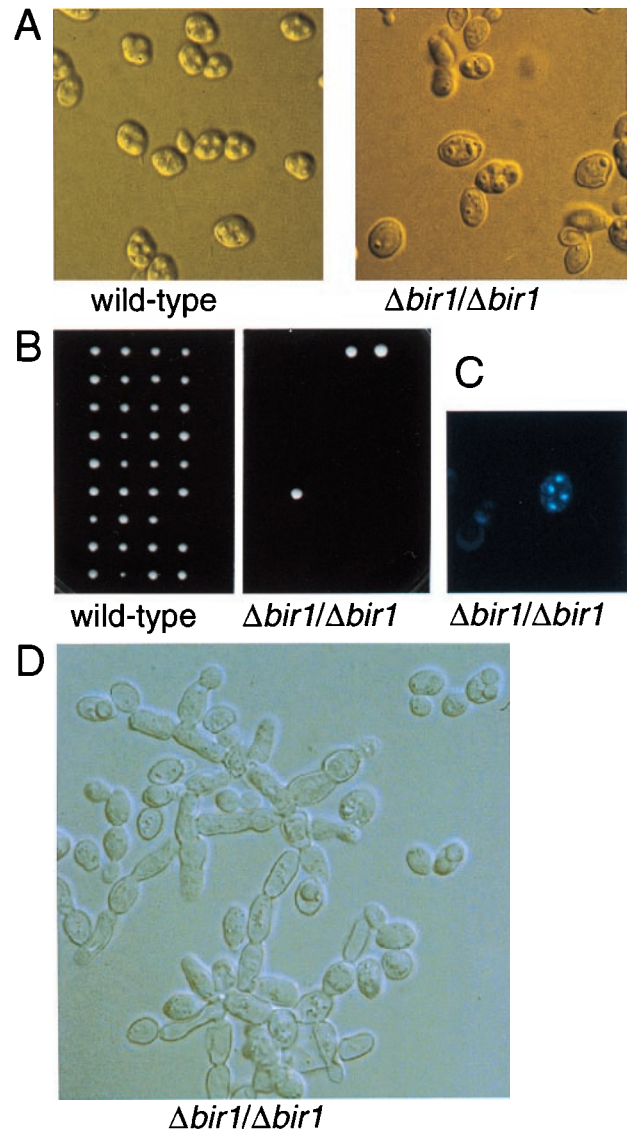
FIG. 2.   Loss of *BIR1* renders diploid *S. cerevisiae* incompetent for sporulation. Wild-type or Δ*bir1*/Δ*bir1* mutant yeast cells were incubated in SM. For wild-type cells, spore formation was almost complete after 3 days (*A Left*). Even after 12 days, few mature spores appeared in the Δ*bir1*/Δ*bir1* culture (*A Right*). (*B*) The few tetrads formed from the Δ*bir1*/Δ*bir1* cells after 12 days on SM were dissected and allowed to germinate and form colonies on rich medium with glucose. (*C*) DAPI staining to visualize the nucleus of each of the spores in the Δ*bir1*/Δ*bir1* tetrads. (*D*) Pseudohyphae in the Δ*bir1*/Δ*bir1* culture after 12 days on SM.

About 10% of cells had undergone either one or two meiotic divisions, and the spores produced within complete tetrads appeared normal (Fig. 3*B*).

Almost half of Δ*bir1*/Δ*bir* cells had a morphology more typical of vegetative cells that have not initiated meiosis, their cytoplasm being more dense with few if any vesicles or lipid bodies, and the vacuole being small if present (Fig. 3*F*). The few (≈3%) cells that had undergone either one or two meiotic divisions looked similar to wild type (Fig. 3*E*).

Bir1 of *S. pombe* Is Required for Spindle Elongation. To examine the function of the *S. pombe bir1* gene, a construct that replaced the ORF of *bir1* with the *ura4* marker by homologous recombination was transformed into both haploid and diploid *S. pombe* cells (Fig. 4*A*). No haploid *bir1::ura4* mutants were obtained, but multiple strains of heterozygous diploid *bir1::ura4/bir1+* strains were isolated that grew and looked normal. When *bir1::ura4/bir1+* heterozygotes were induced to
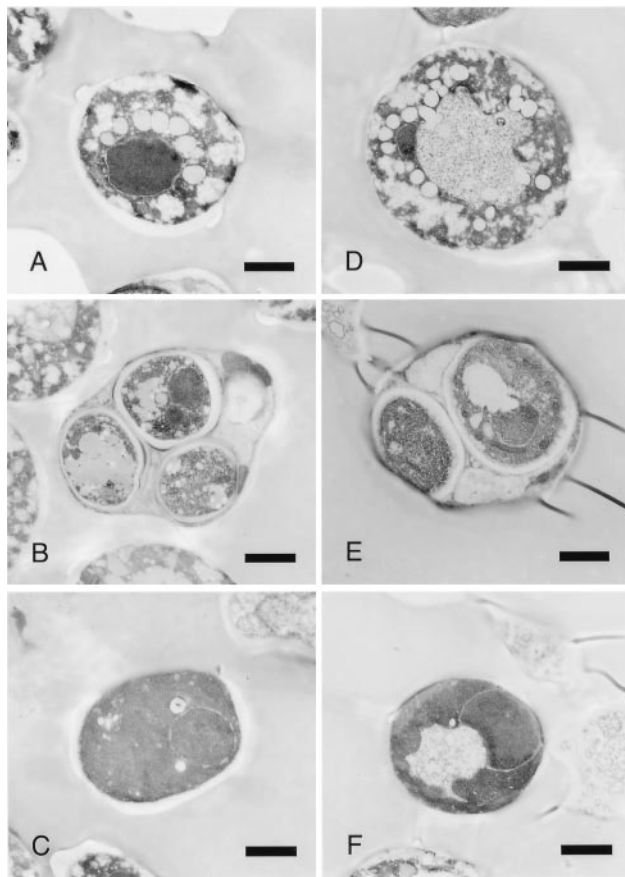
FIG. 3. Electron micrographs of wild-type and (Δ*bir1*/Δ*bir1*) *S. cerevisiae* 28 hr after transfer to SM. (*A*) Typical, highly vacuolated cells, which comprised 81% of the wild-type cells. (*B*) Wild-type asci/spores, which comprised 11% of the wild-type cells, half of which had three or four nuclei. (*C*) Dark, unvacuolated wild-type cells (8% of the population). (*D*) Vacuolated mutant cells (47% of the population). (*E*) Mutant spores comprised 3% of the population, and less than 1% had more than two nuclear profiles. (*F*) Typical, darkly staining *bir1* mutant cells (50% of the mutant cells). (Bar = 1 μm.)

FIG. 4. Failure of the metaphase/anaphase transition in *S. pombe bir1* mutants. (*A*) *bir1* knockout construct consisting of two 1-kb arms of genomic fragments flanking the coding region of *bir1* and a *ura4* marker inserted between these arms. *Hin*dIII sites "H" and *Cla*I sites "C" were used to screen for homologous integrants by Southern blotting. (*B*) Large-scale spore preparations derived from wild-type and *bir1*::*ura4*/*bir1*⁺ heterozygote diploids were germinated in minimal media for 12 hr and stained with DAPI. Wild-type spores germinated normally, with a single, central nucleus in all cells. *bir1*::*ura4* spores germinated but failed to complete mitotic divisions, with septa frequently cutting through the nucleus destroying it or forming on one side of the nucleus creating cytoplasts. (*C*) Tubulin staining of wild-type and *bir1*::*ura4* germinating spores. Cytoplasmic microtubules were present in interphase wild-type cells (*Top Left*). Mitotic cells showing formation and elongation of spindles were present in the culture at normal frequencies. In *bir1*::*ura4* spores (*Right*), microtubules were evident in interphase cells. Mitotic cells possessed only short spindles. In >2,000 cells examined, no evidence for spindle elongation was observed. These cells underwent cytokinesis, often forming a cell plate through the nucleus, killing the cell and, thereby, destroying microtubular structures. (*D*) Bir1-GFP localizes to the nucleus of vegetative *S. pombe*. Green immunofluorescence from Bir1-GFP fusion protein overlaps the DAPI-stained portions of the nucleus. (Bars = 10 μm.)

sporulate, they underwent meiosis and formed asci. Dissection of these asci revealed that only the two wild-type spores from each ascus were viable, whereas the *ura*⁺ mutant spores arrest in cell cycle and/or die during their first or second division after germination.

To study the terminal phenotype of *bir1*::*ura4* cells, we sporulated the heterozygous diploid parent in a liquid culture of minimal medium lacking an added nitrogen source and prepared a pure preparation of spores free of vegetative cells. These were inoculated into minimal medium lacking uracil. Under these conditions, only the *bir1*::*ura4*-containing spores are capable of efficient germination. Samples were taken at various time points after germination, fixed, and stained with DAPI. The *bir1*::*ura4* cells arrested with a single nucleus frequently bisected by a medial septum (Fig. 4*B*). This appearance, termed a "cut phenotype," is characteristic of an inability to successfully complete mitosis before the onset of cytokinesis and is seen in a number of mutants defective in aspects of mitotic progression (reviewed in ref. 25).

In wild-type controls, cells with interphase arrays and mitotic spindles were observed by indirect immunofluorescence. Conversely, *bir1*::*ura4* cells contained only interphase arrays or short mitotic spindles (Fig. 4*C*). As aberrant mitosis progressed, leading to the terminal cut phenotype, microtubule staining was lost, as usually occurs in dead cells. The absence of cells with elongated spindles indicates that the deletion of
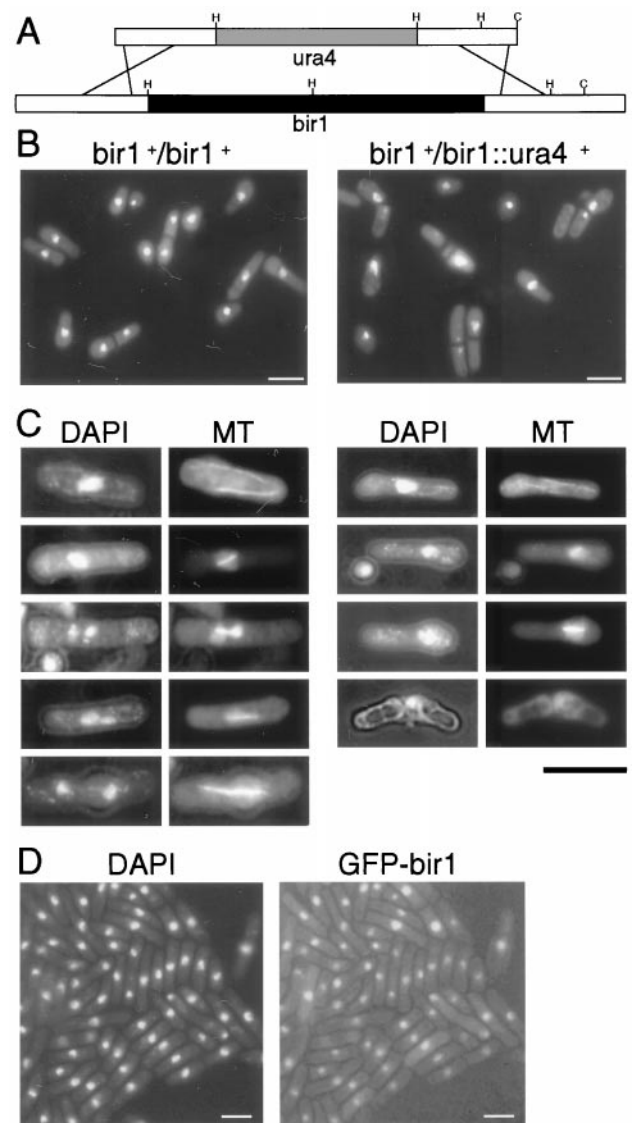
bir1 results in an inability to progress from metaphase to anaphase.

Bir1 Localizes to the Nucleus in *S. pombe* and *S. cerevisiae*. An episomal plasmid construct expressing a green fluorescent

protein (GFP) fused to the C terminus of the *bir1* ORF was transfected into *S. pombe* haploid cells. GFP fluorescence was observed primarily within the nucleus, overlapping the chromatin (Fig. 4*D*). Notably, the transfected cells expressing the highest amounts of bir1-GFP fusion displayed some of the morphological characteristics similar to those seen in the *bir1::ura4* knockout haploids. Furthermore, passaging of bir1-GFP lines frequently resulted in a loss of GFP expression, suggesting that the bir1-GFP fusion may be nonfunctional and, when overexpressed, becomes toxic because of interference with the product of the wild-type allele, or that Bir1 cannot be tolerated at high levels.

In *S. cerevisiae* the case was even more extreme: full-length BIR1-GFP could not be overexpressed in this species of yeast (T.B. and T.L., unpublished data). To determine whether the putative nuclear localization sequence directs BIR1 into the nucleus, we fused domain fragments of the *S. cerevisiae* protein to GFP and ectopically expressed them in *S. cerevisiae* cells grown on rich glucose medium (Fig. 5*A*). BIR1 (1–247)-GFP, a fusion protein bearing the BIR domains alone, remained in the cytoplasm of vegetatively growing yeast cells (Fig. 5*B*). However BIR1(1–474)-GFP, a fusion protein that includes a putative nuclear localization sequence (KKKRKFKR$_{393}$), was found in the nucleus and distributed uniformly through the nucleoplasm (Fig. 5*C*).

The BIR1(249–954)-GFP fusion protein containing the carboxyl-terminal domain of BIR1 also was targeted to the nucleus (Fig. 5*D*), but this protein was associated with a fibrillar structure within the nucleus of dividing cells resembling the spindle of anaphase cells. Treatment of these cells with nocodazole, which disrupts microtubule-base structures, led to a uniform fluorescence of the nucleoplasm (data not shown), suggesting that this domain of the fusion protein can mediate association with spindle fibers.

DISCUSSION

Although the phenotypes of Δ*bir1* mutant *S. cerevisiae* and *bir1* mutant *S. pombe* were not identical, and we have not been able to complement the yeast IAPs with each other or any other IAP, there were similarities between the structure and function of *BIR1* and *bir1*, suggesting that they act similarly in cell division. The primary defect of Δ*bir1 S. cerevisiae* diploid cells was an almost fatal inefficiency in both the first meiotic division of spore formation and the early mitotic divisions after spore germination. Neither Northern analysis of mRNAs nor hybridization of labeled cDNAs to arrays containing the majority of the ORFs of the *S. cerevisiae* genome (*S. cerevisiae* Gene Filters from Research Genetics, Huntsville, AL) revealed clear differences in transcriptional induction or repression between the wild-type and mutant cells during induction of sporulation (data not shown). Despite this normal transcription pattern, more than 90% of the Δ*bir1*/Δ*bir* cells failed to complete the first round of meiotic division, instead remaining as large, rounded diploid cells that had a morphology similar to vegetative cells.

Of the spores that did form from Δ*bir1*/Δ*bir* asci, the majority were inviable. Electron microscopy analysis did not reveal any obvious morphological differences between the few Δ*bir* spores that did form and those from wild-type asci. Although DAPI staining showed there was no gross maldistribution of the DNA, partial aneuploidy because of chromosomal loss during earlier mitotic or meiotic divisions may have occurred.

The Δ*bir1* phenotype closely resembles that of the cell division mutant *chl1* (26). CHL1, a helicase associated with the kinetochore, is required for efficient mitotic chromosome segregation, normal levels of meiotic recombination, and spore viability (26, 27). Diploid *S. cerevisiae* homozygous for *chl1* mutations are viable, but are highly mitotically unstable
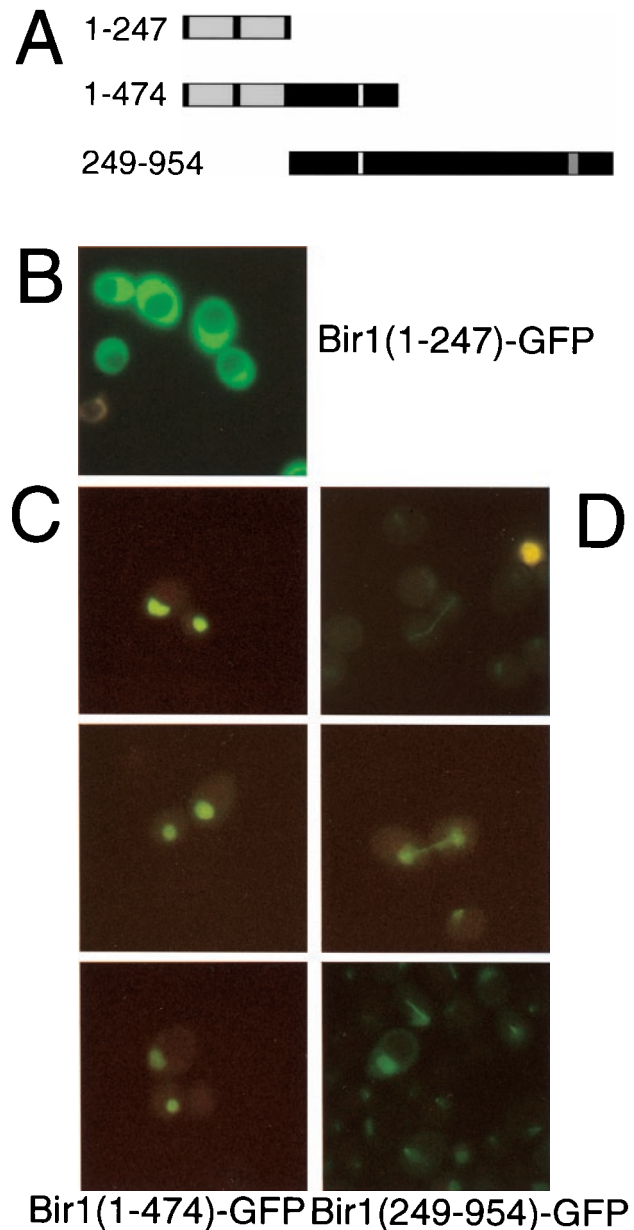

Fig. 5. Expression and nuclear localization of *S. cerevisiae* Bir1-domain fragments fused to GFP. (*A*) Three GFP fusion constructs were designed to carry the BIR domains (shaded), the putative nuclear localization sequence (white), and/or the conserved C-terminal domain (shaded). The yeast strain YAU11 (Δ*bir1*/Δ*bir1*) was transformed with plasmids encoding Bir1(1–247)-GFP (*B*), Bir1(1–474)-GFP (*C*), or Bir1(249–954)-GFP (*D*). Expression of GFP fusions in mid-log-phase cells was analyzed by fluorescence microscopy.

and become aneuploid at 1% per cell division. As a result, spores from *chl1* mutants have low viability because of frequent nullizygosity. Thus, it is possible that BIR1 has a similar role in chromosome segregation during cell division. Such a role is consistent with the nuclear and spindle location of BIR1-GFP.

S. pombe bir1 was required for divisions immediately after germination. Haploid mutant spores derived from *S. pombe bir1::ura4*/*bir1*$^+$ heterozygote diploids were unable to progress more than two divisions after germination. Because these cells die after germination and we were unable to isolate haploid Δ*bir1* mutants by transfecting the knockout construct into haploids, it appears that this gene is also essential for vegetative growth. Unlike the *S. cerevisiae* Δ*bir1*/+ heterozygotes, *S.*

pombe bir1::*ura4*/*bir1*$^+$ heterozygotes underwent normal meiotic division and formed asci that were indistinguishable from wild type. Because these mutants are heterozygotes, however, it is not yet possible to say whether *bir1* also acts in meiosis. The finding that the two wild-type (*ura*$^-$) spores from the asci of *bir1*::*ura4*/*bir1*$^+$ heterozygotes were viable suggests the cell division defects observed in the two *ura*$^+$ spores from each of these asci are cell autonomous.

Tubulin staining of the *S. pombe* haploid cells deleted for *bir1* showed they were capable of undergoing spindle pole body duplication and nucleation of a short metaphase spindle, but were unable to undergo the metaphase-to-anaphase transition. Although cytokinesis proceeded in these cells, a cell plate was formed frequently that cut through the arrested nucleus. Thus, it appears that the lethal phenotype of *bir1* mutants results from an inability to extend the mitotic spindle. The capacity for the C terminus of *S. cerevisiae* BIR1 to localize to the spindle suggests a similar role for the two IAP proteins in spindle function in both species of yeast.

Other IAPs bearing BIRs that resemble those from the yeasts may also have roles in spindle function. Of the vertebrate IAP genes, the sequence and exon structure of the yeast IAPs is most closely related to that of the mammalian IAP Survivin. *survivin* expression increases during the G_2/M phase of cell cycle, and Survivin localizes to the mitotic spindle *in vivo* and cosediments with polymerized tubulin (28). RNA-mediated gene interference of one of the IAPs from *C. elegans*, caused abnormalities in cytokinesis during embryonal cell divisions (11).

Although it is not clear whether vertebrate and insect IAPs function primarily by blocking caspase activation signals (29–31) or by binding directly to caspases (32–35), most are thought to inhibit a caspase-dependent apoptotic mechanism. Survivin has been reported to bind directly to, and inhibit, caspases 3 and 7 (35), but its homologs in *C. elegans*, *bir-1* and *bir-2*, appear to have no role in the control of cell death, and the phenotype caused by inactivation of one of the *C. elegans* IAPs can be suppressed partially by transgenic expression of *survivin* (11). Because neither *S. pombe* nor *S. cerevisiae* appears to encode caspases, and neither has been shown to use a cell suicide program, Bir1 and BIR1 are unlikely to function by inhibiting cell death mechanisms resembling those in metazoans.

The structural and functional similarities between human Survivin, *cerevisiae* BIR1, *pombe* Bir1, and the BIR-bearing proteins from *C. elegans* suggest that they share conserved roles in cell division.

1. Vaux, D. L. & Strasser, A. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 2239–2244.
2. Vaux, D. L. & Korsmeyer, S. J. (1999) *Cell* **96,** 245–254.
3. Uren, A. G., Coulson, E. J. & Vaux, D. L. (1998) *Trends Biochem. Sci.* **23,** 159–162.
4. Birnbaum, M. J., Clem, R. J. & Miller, L. K. (1994) *J. Virol.* **68,** 2521–2528.
5. Crook, N. E., Clem, R. J. & Miller, L. K. (1993) *J. Virol.* **67,** 2168–2174.
6. Hawkins, C. J., Uren, A. G., Hacker, G., Medcalf, R. L. & Vaux, D. L. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 13786–13790.
7. Duckett, C. S., Nava, V. E., Gedrich, R. W., Clem, R. J., Vandongen, J. L., Gilfillan, M. C., Shiels, H., Hardwick, J. M. & Thompson, C. B. (1996) *EMBO J.* **15,** 2685–2694.
8. Hay, B. A., Wassarman, D. A. & Rubin, G. M. (1995) *Cell* **83,** 1253–1262.
9. Liston, P., Roy, N., Tamai, K., Lefebvre, C., Baird, S., Cherton-horvat, G., Farahani, R., Mclean, M., Ikeda, J. E., Mackenzie, A., *et al.* (1996) *Nature (London)* **379,** 349–353.
10. Uren, A. G., Pakusch, M., Hawkins, C. J., Puls, K. L. & Vaux, D. L. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 4974–4978.
11. Fraser, A. G., James, C., Evan, G. I. & Hengartner, M. O. (1999) *Curr. Biol.* **9,** 292–301.
12. Dawes, I. W. & Hardie, I. D. (1974) *Mol. Gen. Genet.* **131,** 281–289.
13. Wach, A., Brachat, A., Albertisegui, C., Rebischung, C. & Philippsen, P. (1997) *Yeast* **13,** 1065–1075.
14. Blacketer, M. J., Koehler, C. M., Coats, S. G., Myers, A. M. & Madaule, P. (1993) *Mol. Cell. Biol.* **13,** 5567–5581.
15. Gavrias, V., Andrianopoulos, A., Gimeno, C. J. & Timberlake, W. E. (1996) *Mol. Microbiol.* **19,** 1255–1263.
16. Moreno, S., Klar, A. & Nurse, P. (1991) *Methods Enzymol.* **194,** 795–823.
17. Craig, R. & Norbury, C. (1998) *J. Cell Sci.* **111,** 3609–3619.
18. Forsburg, S. L. (1993) *Nucleic Acids Res.* **21,** 2955–2956.
19. Krien, M. J. E., Bugg, S. J., Palatsides, M., Asouline, G., Morimyo, M. & O'Connell, M. J. (1998) *J. Cell Sci.* **111,** 967–976.
20. Ambrosini, G., Adida, C. & Altieri, D. C. (1997) *Nat. Med.* **3,** 917–921.
21. Hauser, H. P., Bardroff, M., Pyrowolakis, G. & Jentsch, S. (1998) *J. Cell Biol.* **141,** 1415–1422.
22. Kron, S. J., Styles, C. A. & Fink, G. R. (1994) *Mol. Biol. Cell* **5,** 1003–1022.
23. Lithgow, T., Junne, T., Suda, K., Gratzer, S. & Schatz, G. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 11973–11977.
24. Chu, S., Derisi, J., Eisen, M., Mulholland, J., Botstein, D., Brown, P. O. & Herskowitz, I. (1998) *Science* **282,** 699–705.
25. Yanagida, M. (1998) *Trends Cell Biol.* **8,** 144–149.
26. Liras, P., McClusker, J., Mascioli, S. & Haber, J. E. (1978) *Genetics* **88,** 651–671.
27. Amann, J., Kidd, V. J. & Lahti, J. M. (1997) *J. Biol. Chem.* **272,** 3823–3832.
28. Li, F. Z., Ambrosini, G., Chu, E. Y., Plescia, J., Tognin, S., Marchisio, P. C. & Altieri, D. C. (1998) *Nature (London)* **396,** 580–584.
29. Manji, G. A., Hozak, R. R., Lacount, D. J. & Friesen, P. D. (1997) *J. Virol.* **71,** 4509–4516.
30. Vucic, D., Kaiser, W. J., Harvey, A. J. & Miller, L. K. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 10183–10188.
31. Vucic, D., Kaiser, W. J. & Miller, L. K. (1998) *Mol. Cell. Biol.* **18,** 3300–3309.
32. Deveraux, Q. L., Takahashi, R., Salvesen, G. S. & Reed, J. C. (1997) *Nature (London)* **388,** 300–304.
33. Takahashi, R., Deveraux, Q., Tamm, I., Welsh, K., Assamunt, N., Salvesen, G. S. & Reed, J. C. (1998) *J. Biol. Chem.* **273,** 7787–7790.
34. Roy, N., Deveraux, Q. L., Takahashi, R., Salvesen, G. S. & Reed, J. C. (1997) *EMBO J.* **16,** 6914–6925.
35. Tamm, I., Wang, Y., Sausville, E., Scudiero, D. A., Vigna, N., Oltersdorf, T. & Reed, J. C. (1998) *Cancer Res.* **58,** 5315–5320.